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Dear Leon,

I have held off writing to you until I could accumulate some data that would be interesting and convincing. First let me deal with the properties of the yeast RNA you sent (Monier prep.):

1. When tested for its ability to accept leucine and valine with the purified enzymes it was essentially inert (Purified coli RNA accepts 0.83 and 1.0 mμmoles per 10 optical density units of C^{14} valine and C^{14} leucine respectively while the yeast RNA was <0.01 with each).

2. If this RNA was first reacted with ATP and CTP and the A- C-incorporating enzyme there was no increase in the ability to accept valine and leucine.

3. Measurement of the amount of A and C which can be linked to the RNA was 9.5 mμmoles of A and 2.5 mμmoles of C per 10, OD units. This suggests that the RNA has most of the A ends removed and only a few of C residues removed. This I believe is consistent with what you told me of the results of your end group analysis of this material. By contrast the purified "native" coli RNA accepts very little A and C (<0.5 mμmoles of A or C per 10 OD units).

I am quite curious as to whether you have tested the Monier RNA for its ability to accept amino acids with the mammalian enzymes and whether this requires the addition of CTP to the reaction mixture. The requirement for A addition is not easy to determine if one uses a "pH 5 enzyme" since in the presence of ATP for the assay AMP would be added. If this RNA prep. is active with mammalian enzymes but not the coli enzymes this would be extremely interesting.

Now with regard to the action of the Goren-Levinthal phosphatase. You remember that while you were here we did one experiment to determine whether incubation of coli RNA with BAP resulted in loss of biological activity. The following is a summary of the results of that first experiment.

	Amino Acid Acceptor Activity %		Pyrophosphorolysis of A and C ends %
	leucine	valine	
A (No enzyme, incubated at 60° for 120 min.)	62	51	46
B (BAP 6003 B (20 μg/mg RNA) incubated at 60° for 120 min.)	6	<5	<5
C (BAP 6005 (13 μg/mg RNA) incubated at 60° for 120 min.)	<5	<5	<5
D (No enzyme, 0° for 120 min.)	100	100	100

Thus incubation with BAP inactivated the RNA for all activities to a much greater extent than due to the non-enzymic inactivation. We repeated this experiment at 60° and at 37° (using the DNA-free purified RNA) taking samples at various times for measurement of P_i release and valine-acceptor activity. The resul The results are summarized on the enclosed sheet. Several things are to be noted:

1. At 60° where P_i release essentially came to a halt (ca. 2% increase between 60 - 120') the apparent end group value is 1 in 36 which I'm sure is incorrect. Under these conditions the valine-acceptor activity is essentially completely lost by 120'. If one corrects for non-enzymatic decay of activity one finds that by the time 70% of the phosphate release has occurred only 25% of the activity has been lost. The remaining loss of activity accompanies the liberation of only the last 30% of the P_i. I interpret this as indicating:

   a. The removal of the terminal P may not result in inactivation of activity. Assuming a chain length of 91 (based on nucleoside end groups) all the terminal P should have represented 0.187 μmole P_i at completion. At this point one can estimate that very little activity has been lost (assuming terminal P comes off first).

   b. The excess P_i is due to nuclease action exposing new end groups and loss of acceptor activity. This nuclease activity would have to average about 1.5 hits per chain per 120'.

2. At 37° the phosphate release is slower (it doesn't plateau) but it still exceeds the value for 1/91 by about 1.8 x. Interpreting this as a nuclease we would have to say that statistically there is 0.8 hits per chain per 120'.

Assuming that all chains are equally susceptable to this hypothetical nuclease one can calculate the fraction of chains which have received no hits, e.g. with an average of 1.5 hits/chain $C^{-1.5}$ = fraction of chains with no hits = 0.22. Thus 22% of the chains would be expected to be intact and we find 20% of valine activity remaining (correcting for non-enzymic inactivation). In the 37° incubation

there have been 0.8 hits per chain or $C^{-0.8}$ = surviving fraction = 0.45 or 45% intact chains. This again equals the remaining activity.

All in all if one makes the assumption that the kinetics of P_i release represent a fast elimination of the terminal P and a slower release of P due to cleavage of chains internally then we could say that in both cases essentially all of the terminal P is removed by 5 - 15 minutes and yet there is little inactivation of acceptor activity. The remaining P_i coming from internal breaks should then parallel loss in activity. This as you can see is roughly the case.

One of the major things which puzzles me about this is that with the yeast RNA (Monier) you get 1 P released per 80 total P and it seems stable, i.e. as if no slow attack by nuclease. There are lots of wild suggestions one could make but they don't seem worthwhile at present. I did do one experiment to determine if we could have had a trace (1 - 2%) of mononucleotides present which could have remained after dialysis following treatment of our RNA with Lehman's diesterase. This contaminant of 5'-nucleotides would have been roughly equal to the amount of P liberated from the end group. However treatment with a purified 5'-nucleotidase gave no P_i so this is ruled out and I'm convinced the extra P is coming from the RNA.

That's all we've done on this so far. We are still doing the "sequence" study for valyl and leucyl-specific RNA chains and hope to know what the 4th and perhaps 5th and 6th nucleotides are. I'll let you know later. What kind of progress have you all made? I still expect to be in Bethesda December 9, but I'm not sure of the exact days. I'll let you know later on this too.

Give my best to Jack, Russ, Gil, Maxine and all the rest of your group.

With best regards from all here

Sincerely

Paul Berg

PB:cm